

Evaluation of the Bactec MGIT 960 System in Combination with the MGIT TBc Identification Test for Detection of *Mycobacterium tuberculosis* Complex in Respiratory Specimens[▽]

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Received 22 March 2011/Accepted 22 March 2011

The sensitivity and specificity of the MGIT TBc identification (TBc ID) test for *Mycobacterium tuberculosis* complex (MTC) detection in positive Bactec MGIT cultures were 95.2% and 99.2%, respectively. When MTC-positive results obtained from two additional molecular methods were included, the sensitivity of the MGIT TBc ID test was 85.4%, while that of culture was 95.7%.

The Bactec MGIT 960 liquid culture system (Becton, Dickinson and Company) can shorten the time of recovery of mycobacteria to approximately 10 to 14 days (5, 13). Even though liquid culture reduces the time to detection, labor-intensive biochemical methods for species identification in positive cultures are needed. The MGIT TBc identification (TBc ID) test (Becton, Dickinson and Company), based on the detection of a protein (MPT64) secreted by *Mycobacterium tuberculosis* complex (MTC), can detect MTC in 15 min from acid-fast bacillus (AFB)-positive MGIT cultures. However, mutation, insertion, and deletion of the *mpb64* gene and insufficient growth of MTC in liquid culture can result in false negatives (3, 4, 9, 10). Furthermore, strains of some nontuberculous mycobacteria, such as *M. avium* complex (12, 16), *M. chelonae* (16), and *M. marinum* and *M. flavescentis* (1), can produce false positives. We aimed to evaluate the TBc ID test for MTC detection in AFB-positive MGIT cultures from respiratory specimens.

A total of 3,832 respiratory specimens (3,535 sputum, 237 bronchial aspirate, 56 bronchoalveolar lavage fluid, and 4 lung abscess samples) from 1,324 patients with suspected tuberculosis (TB) were tested in order to exclude the possibility of other mycobacterial infections. The study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital. Each specimen was inoculated into a Löwenstein-Jensen (LJ) tube and an MGIT 960 tube (7). AFB smears from growth-positive MGIT tubes were performed. Subculture was made from the AFB-positive tubes onto LJ and 7H11 media. An aliquot (0.1 ml) of AFB-positive MGIT broth was analyzed by the TBc ID test. Mycobacteria isolated from culture were

identified by morphological, biochemical, and PCR-restriction fragment length polymorphism (RFLP) analysis (14, 15). An aliquot (0.2 ml) of AFB-positive MGIT broth was used to prepare a DNA template for the GenoType Mycobacterium CM assay (Hain Life Science GmbH, Germany).

When discrepant results between culture and the TBc ID test occurred, additional determinations of MTC infection were made with the Cobas TaqMan MTB assay (Roche Diagnostics, Taipei, Taiwan) and the GenoType Mycobacterium CM assay and by a review of the patient's medical history. Normally, the TaqMan MTB test was used for AFB smear-positive specimens (6); smear-negative specimens were analyzed if the test was ordered by a physician. Each patient's medical history was classified into one of five groups as recommended previously (2).

Of the 3,832 specimens, 755 were MGIT growth positive. Among these, 291 samples were AFB positive. From the 291 AFB-positive cultures, the TBc ID assay yielded 274 (94.2%) results (150 MTC positives and 124 MTC negatives) concordant with culture results. The TBc ID test yielded nine false positives and eight false negatives. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the TBc ID test were 94.9%, 93.2%, 94.3%, and 93.9%, respectively, before discrepancies were resolved. The mean duration from receipt of specimens to report of MTC with culture method (28.3 days) was about double that obtained with the TBc ID test (13.3 ± 8 days).

Of the nine false-positive samples by the TBc ID test, eight were true positives, as MTC was detected in the MGIT cultures by the GenoType Mycobacterium CM assay, and four of these were also TaqMan MTB test positive (Table 1). Medical history review indicated that the eight specimens were from TB patients. Specimen T03951 was MTC positive by three assays (TBc ID, GenoType Mycobacterium CM, and TaqMan MTB), but *M. abscessus* was detected by culture and the GenoType Mycobacterium CM test. It seemed that specimen T03951 was

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[▽] Published ahead of print on 30 March 2011.

TABLE 1. Analysis of discrepancies in MTC detection from 36 respiratory specimens (31 patients)

Specimen no. (AFB stain)	MGIT culture (growth/AFB stain)	Detection of MTC (other mycobacteria) by:				Medical history group ^b	Final interpretation of TBc ID test
		TBc ID test	Culture ^a	GenoType Mycobacterium CM test ^a	Cobas TaqMan MTB test		
T03217 (+), T06197 (+), T06218 (+)	+/+	+	—	+	+	5	True positive
T03951 (+)	+/+	+	— (<i>M. abscessus</i>)	+	+	4	True positive
T05086 (—)	+/+	+	— (unidentified mycobacterium)	— (<i>M. kansasii</i>)	ND ^c	1	False positive
T05093 (—)	+/+	+	—	+	ND	5	True positive
T05179 (—)	+/+	+	—	+	ND	4	True positive
T05525 (—)	+/+	+	— (<i>M. gordonae</i>)	+	ND	4	True positive
T05968 (—)	+/+	+	—	+	ND	3	True positive
T03271 (+), T04786 (+), T05211 (+), T05694 (+)	+/+	—	+	+	+	5	False negative
T04270 (+)	+/+	—	+	—	—	5	False negative
T03960 (—)	+/+	—	+	+	—	4	False negative
T03997 (—), T05087 (—)	+/+	—	+	+	+	4	False negative
T03794 (+), T04060 (+), T05044 (+), T05089 (+), T06165 (+), T07021 (+)	+/-	ND	+	+	+	4	False negative
T04400 (—), T04839 (—), T04916 (—), T07164 (—)	+/-	ND	+	—	ND	4	False negative
T06443 (+)	+/-	ND	+	+	+	4	False negative
T03331 (—), T03893 (—)	+/-	ND	+	+	+	4	False negative
T03577 (—), T05105 (—), T05337 (—)	+/-	ND	+	+	ND	4	False negative
T04700 (—), T04723 (—), T04788 (—)	+/-	ND	+	+	+	4	False negative

^a Nontuberculous mycobacteria detected are listed in parentheses.

^b See reference 2 for descriptions of medical history groups.

^c ND, not determined, when the Cobas TaqMan MTB test was not ordered by the physician or the TBc ID test was not performed if there was no growth in the MGIT tube or the tube was growth positive but AFB negative.

a mixed culture of MTC and *M. abscessus*. A similar situation was found for specimen T05525, which contained MTC and *M. gordonae*. The only false-positive sample by the TBc ID test was specimen T05086; *M. kansasii* was detected in this sample by the GenoType Mycobacterium CM assay (Table 1).

The TBc ID test yielded eight false negatives, since MTC was isolated by culture. We sequenced the *mpb64* gene of each of these eight isolates (8). Three isolates, from a single patient, had the same mutation, an insertion of one base (C) at nucleotide position 573 that made a frameshift of the *mpb64* gene. This insertion mutation has not been reported previously (3, 4, 10, 17). A possible explanation for the other five false negatives was that the MTC cell numbers in the MGIT tubes were too low to produce sufficient MPT64 antigen for detection. It has been estimated that at least 10⁵ CFU/ml of MTC is needed to produce a positive reaction by a lateral-flow chromatographic test for MTC detection (11, 17).

After discrepancies were resolved, the sensitivity, specificity, PPV, and NPV of the TBc ID test were 95.2%, 99.2%, 99.4%, and 93.9%, respectively. The sensitivity (95.2%) of the TBc ID test was comparable to those (96.9% to 99.6%) reported for the Capilia TB assay (Tauns, Numazu, Japan), another immunochromatographic assay for MTC detection in liquid culture (8–12). Yu et al. (17) evaluated the TBc ID test and reported a sensitivity rate of 98.8%. However, growth-positive but AFB-negative MGIT cultures and TBc ID test-positive but culture-negative specimens were not analyzed further in that study. The specificity of the TBc ID test after discrepant analysis approached 99.2%. The high specificity may reduce unnecessary antituberculosis treatment and subsequent adverse effects.

Nineteen specimens that were growth positive but AFB negative in the MGIT tubes were found to contain MTC by culture (Table 1). Normally, these tubes were not analyzed by the TBc ID test, since the test was recommended only for growth- and AFB-positive MGIT cultures by the manufacturer. If specimens that were MTC positive by all methods (LJ culture, 7H11 culture, TBc ID test, GenoType Mycobacterium CM assay, and TaqMan MTB test) are summed together, 185 specimens (166 from AFB-positive and 19 from AFB-negative MGIT cultures) were positive for MTC. Then, the sensitivity of the MGIT 960 system-TBc ID test combination was 85.4% (158/185), while that of culture was 95.7% (177/185).

In conclusion, the MGIT 960 system-TBc ID test combination reduces the turnaround time of MTC diagnosis. However, this combination is not as sensitive as the traditional culture method. The integration of solid and liquid culture and an additional molecular method, such as the TaqMan MTB or GenoType Mycobacterium CM assay, can yield a sensitivity approaching 100% for MTC detection.

This project was supported by grants from the National Science Council (NSC 99-2321-B-006-007), Kaohsiung Medical University Hospital, and the Multidisciplinary Center of Excellence for Clinical Trial and Research (DOH100-TD-B-111-102), Department of Health, Executive Yuan, Taiwan.

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